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EVALUATION OF PATHOLOGICAL VARIABILITY BY SCREENING OF *VERTICILLIUM FUNGICOLA* ISOLATES WITH DIFFERENT MUSHROOMS SPECIES

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ABSTRACT

Verticillium fungicola is a serious pathogen causing dry bubble disease in button mushroom (Agaricus bisporus). Present investigations were carried out on both host and pathogen by covering an aspect of variability in isolates of pathogen in terms of pathogenicity. The V. fungicola isolates collected from different geographical regions of Haryana, isolated, purified on PDA medium and coded as MHS (Hisar), BFT (Fatehabad), NJN (Jind), RHT (Rohtak), TPN (Panipat), BSN (Sonipat), FDB (Fridabad) and SKK (Kurukshetra) and pathogenicity was proved on host A. bisporus. Regarding pathogenic variability among the isolates, during screening of the isolates, only BSN, TPN, FDB, SKK and RHT showed disease symptoms on fruiting bodies of all the strains of A. bisporous included in the study and other mushroom spp. i.e. A. bitorquis, Pleurotus sajor-caju and P. florida, except A. bisporous strain U-3 and Calocybe indica where no disease appeared

KEYWORDS: Verticillium fungicola, Agaricus Bisporus, Isolate & Variability

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Original Article

INTRODUCTION

The commercial production of edible mushroom converts different types of agricultural and house-hold wastes into nutrition rich food which helps in addressing the problems of quality food, health and environmental sustainability. In view of increasing demand of high quality food with an increasing world population, mushrooms will be an important source of proteins that can replace meat and vegetables and milk products for a major part (Wani *et al*., 2010). About 1.5 million species of fungus are known and out of these it has been estimated that 14,000 species produce fruiting bodies that are desirable to be considered as mushrooms (Hawksworth, 2001). About 7,000 species of edible mushrooms are known out of which 200 are experimentally grown and 10 have been produced at the industrial scale (Chang and Miles, 2004).

Presently, three geographical regions *viz.*, Europe, America and East Asia contribute 96 per cent of world mushroom production. India contributes 4.59 lakh metric tons, which is 3 per cent of total world production and in Haryana; the annual mushroom production is about 10,207 metric tons (Anonymous, 2017). Button mushroom is a popular and valuable food, low in calories, fat and high in essential amino acids, vitamins and fibers and supply a range of valuable minerals especially potassium and iron (Mattila *et al*., 2002).

In India, mostly four species of edible mushrooms *viz*., *Agaricus bisporus* (white button mushroom), *Volvariella* spp. (paddy straw mushroom), *Pleurotus* spp. (oyster mushroom) and *Calocybe indica* (milky mushroom) are commercially cultivated. Mushroom cultivation is affected by a large number of biotic and abiotic factors. Fungi, bacteria, viruses, nematodes, insects and mites are different biotic factors that damage the mushroom crop directly or indirectly (Sharma *et al*., 2011). Among the various factors responsible for low production and productivity of mushroom in our country, fungal diseases play a major role. The fungal pathogens, *Verticillium fungicola*, *Mycogone perniciosa*, *Trichoderma* spp. and *Papulaspora byssina* are the predominant mycopathogens. Amongst these, *Verticillium fungicola* var. *fungicola* (Preuss) is the important pathogen of the *Agaricus bisporus* (Lange) Imbach and annual losses to the growers are estimated to be 2–4% of total revenue (Berendsen *et al*., 2010). The pathogen induces various symptoms like bubbles (undifferentiated spherical masses), bent and/or split stipes (blowout) and spotty caps. Inoculation of *A. bisporus* crop with isolates of *V. fungicola* var. *fungicola* of various degrees of aggressiveness showed that the more aggressive isolates induced higher numbers of bubbles (Largeteau and Savoie, 2008). The *Verticillium* dry bubble is the most prevalent disease and if left uncontrolled in the mushroom growing environment; the disease can wipe out an entire crop in 2–3 weeks (Sharma *et al*., 2002).

Dry bubble was first detected in a commercial planting in North America in 1981. However, literature on dry bubble has been published in India as early as 1960, proving that it has been an economic problem for mushroom growers during 20^{th} century. It mainly affects three different species of mushrooms *viz. Agaricus bisporus*, *A. bitorquis* and *Pleurotus ostreatus*. Though, the infection by *V. fungicola* does not decrease the weight of the mushrooms, but has the potential to decrease the total number of mushrooms produced (Berendsen *et al*., 2010).

Meagre information is available on *V. fungicola* in India, especially regarding pathological variability studies, for effectively management of disease. Therefore, keeping this in view; the present investigations were undertaken with the objectives of pathogenic variability of *Verticillium fungicola* isolates.

MATERIALS AND METHODS

The present investigation entitled "pathogenic variability of *Verticillium fungicola* isolates collected from different mushroom farm of Haryana state" was carried out at Mushroom Technology Laboratory, Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, during 2015-2017 seasons. Hisar is situated at a latitude of $29^{o}10$'N, longitude 75^{o} 46'E and an altitude 215.2 m above mean sea level and fall in semi-tropical regions of Western Zone of India. Details of the materials used and methodology adopted during the course of this investigation are given below.

Pathogenic Variability of *V. Fungicola* Isolates

In the present studies, 17 diseased samples in triplicate were collected from different parts of the Haryana, out of these *V. fungicola* was isolated from eight samples. Samples were washed thoroughly with tap water, dried and then kept in paper bags for further isolation of pathogen. The fungi were cultured on fresh potato dextrose agar medium for isolation, purification and pathogenicity test. The pathogen was purified and maintained by repeated sub-culturing of each isolates after every month and kept in a refrigerator at 4°C for the further studies. Isolation of the *V. fungicola* was made from the infected fruiting bodies, which showing typical symptoms of dry bubble disease. The diseased fruiting bodies were first examined for the associated pathogen by teasing the diseased portion with the help of a needle and observed under

microscope. For isolation of the causal fungus, five mm small disc segments were cut from the infected sporophore with the help of sterilized cork borer, surface sterilized with 0.1% mercuric chloride for 30 seconds followed by rinsing thrice with sterilized distilled water, blotter dried and inoculated under aseptic conditions on PDA medium in sterilized Petri dishes and incubated at 25±1°C (Sabharwal and Kapoor, 2014).

The pathogen culture was purified by hyphal tip culture method (Pathak, 1972). The pure culture was obtained and maintained by repeated sub-culturing at monthly intervals. The stock culture in PDA slants was stored at 4±1°C in a refrigerator. The repeated sub-culturing was done for further studies to avoid the possible loss of pathogenic behavior of the test pathogen. The studies of screening of *V. fungicola* isolates with *A. bisporous* (strains S-11, U-3 and AICRP on mushroom coded ABL-1, ABL-2, ABL-3, ABL-4, ABL-5, ABL-6 and ABL-7), *A. Bitorquis*, *Pleurotus sajor-caju*, *P. florida* and *Calocybe indica* were carried out by cut fruit body inoculation technique. The uniform spore suspension (2×10^6 ml^{-1}) of *V. fungicola* isolates were inoculated on cut fruiting bodies of different mushrooms and incubated in an isolated room at a temperature 25±1°C with high relative humidity (>85%) and the symptoms appearance were recorded in different mushrooms and their strains.

RESULTS AND DISCUSSIONS

During variability studies in *V. fungicola*, the 17 diseased samples of white button mushroom were collected from different parts of the Haryana and from these *V. fungicola* was isolated from eight samples. Isolation of *V. fungicola* was done from the diseased samples and pathogenicity was proved on white button mushroom. The cultures were purified and maintained on potato dextrose agar medium. The purified *V. fungicola* isolates were coded (Table-1, Plate-1) as MHS (Hisar), BFT (Fatehabad), NJN (Jind), RHT (Rohtak), TPN (Panipat), BSN (Sonipat), FDB (Fridabad) and SKK (Kurukshetra). The studies of screening of *V. fungicola* isolates with *A. bisporous* (strains S-11, U-3 and AICRP on mushroom coded as ABL-1, ABL-2, ABL-3, ABL-4, ABL-5, ABL-6 and ABL-7), *A. bitorquis*, *Pleurotus sajor-caju*, *P. florida* and *Calocybe indica* were carried out by cut fruit body inoculation technique (Table 2, Plate-1). It was observed that isolates BSN, TPN, FDB and RHT infected and showed symptoms in almost all the screened strains of *A. bisporous* and other mushroom spp. *i.e. A. bitorquis, Pleurotus sajor-caju* and *P. florida* except *A. bisporous* strain U-3 and *Calocybe indica*. On the other hand, *Pleurotus sajor-caju* and *A. bisporous* strains ABL-1 and ABL-5 were infected by all the screened *V. fungicola* isolates. In pathological variability studies carried out on cut fruiting bodies of different strains of *A. bisporous* and other mushrooms spp. it was observed that isolates BSN, TPN, FDB and RHT were more virulent and showed symptoms almost in all *i.e. A. bitorquis, Pleurotus sajor-caju* and *P. florida*, except *A. bisporous* strains U-3 and *Calocybe indica*. Similarly, our finding are in complete agreement with work of Gea *et al*. (2003) who observed that *V. fungicola* infect mushrooms such as *A. bisporus*, *A. bitorquis* and *Pleurotus* spp. On the other hand, Berendsen *et al*. (2010) reported that *V. fungicola* produced disease symptoms mainly in three different spp. of mushrooms *viz. Agaricus bisporus*, *A. bitorquis* and *Pleurotus ostreatus*. On the other hand, Amey *et al*. (2007) observed that *V. fungicola* was also closely associated with a variety of insect pathogens therefore, it was suggested that it also able to infect pest.

SUMMARY AND CONCLUSIONS

The pathogenic potential of *V. fungicola* isolates was determined on the basis of symptoms appearance on fruiting bodies of different mushrooms species. During screening it was observed that isolates BSN, TPN, FDB and RHT could infect fruiting bodies in almost all the screened strains of *A. bisporous* and other mushroom species *i.e. A. bitorquis*,

Pleurotus sajor-caju and *P. florida* except *A. bisporous* strain U-3 and *Calocybe indica*, where no disease symptoms appeared. Isolate BSN proved to be highly virulent. Besides this, button mushroom (*A. bisporus* and its strains) and other mushroom spp. (*A. bitorquis*, *Pleurotus sajor-caju* and *P. Florida*) are also infected by dry bubble causing *V. fungicola* pathogen.

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Table 1: Different Isolates of *V. fungicola* Pathogen Collected from Different Geographical Regions

Isolates of *Verticillium fungicola*	White Button Mushroom Diseased Samples* Collection		
	Isolates Code	Village/ City	District
VF-1	MHS	Matarshyam	Hisar
VF-2	BFT	Bhattu	Fatehabad
VF-3	NJN	Narwana	Jind
VF-4	RHT	Rohtak	Rohtak
VF-5	TPN	Taharpur	Panipat
VF-6	BSN	Bainyapur	Sonipat
VF-7	FDB	Fridabad	Fridabad

VF-8	SKK	Sudha mushroom lab, Kurukshetra	Kurukshetra

***** Average of three replications

Table 2: Evaluation of Pathological Variability by Screening of Different Mushroom Strains with Different Isolates of *V. Fungicola*

Sr. No.	*V. Fungicola* Isolates Code	Site of Isolates Collection	*Observation on Symptoms Development of Dry Bubble Disease in Different Mushroom Strains by Fruit Body Inoculation Technique										
			Agaricus Bisporus Strains		*Agaricus bisporus* AICRP ABL coded strains						*Agaricus Bitorquis*	*Pleurotus Spp.*		*Calocybe Indica*
			Strains S-11	Strains U-3	1	2	3	4	5	6	7		*P. Sajor-Caju*	*P. Florida*	
1	MHS	Hisar	+	-	+	-	+	+	+	-	+	-	+	+	-
2	BFT	Fatehabad	-	-	+	+	-	-	+	+	-	+	+	-	-
3	JNJ	Jind	+	-	+	-	+	+	+	-	+	+	+	-	-
4	RHT	Rohtak	-	-	+	+	+	+	+	+	+	+	+	+	-
5	TPN	Panipat	+	-	+	+	+	+	+	+	+	+	+	+	-
6	BSN	Sonipat	+	-	+	+	+	+	+	+	+	+	+	+	-
7	FDB	Fridabad	+	-	+	+	+	+	+	+	+	+	+	+	-
8	SKK	Kurukshetra	+	-	+	-	+	+	+	-	+	-	+	-	-
9	DMR-Reference	DMR-Solan	+	-	+	+	+	+	+	+	+	+	+	+	-

***** Average of three replications, Where (+) symptoms present and (-) symptoms absent

Plate-1

Figure 1: *Verticillium fungicola* isolates Collected from Different Locations

Figure 2: Screening of Different Mushroom Strains with *Verticillium Fungicola* Isolates